

Monocyte-derived chicken macrophages exposed to *Eimeria tenella* sporozoites display reduced susceptibility to invasion by *Toxoplasma gondii* tachyzoites

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Short report

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Abstract

Background: Both *Eimeria (E.) tenella* and *Toxoplasma (T.) gondii* are common apicomplexan parasites in chickens. Host cell invasion by both protozoans includes gliding motility, host cell attachment and active penetration. Chicken macrophages as phagocytic cells participate in the innate host immune response against these two parasites.

Methods: In this study, primary chicken monocyte-derived macrophages (MMs) were infected with both pathogens to investigate mutual and host-parasite interactions. MMs cultures were assigned to groups that were infected with *E. tenella*, *T. gondii*, or both. In co-infected cultures, MMs were first exposed to *E. tenella* sporozoites for 2 hours. Afterwards, *T. gondii* tachyzoite infection was performed. Live-cell imaging was carried out to observe cell invasion and survival of *T. gondii* by single parasite tracking over a period of 20 hours post infection (hpi). Quantitative analysis for parasite replication was performed at 2, 6, 12 and 24 hpi by real-time PCR (qPCR).

Results: We found that during early co-infection *T. gondii* tachyzoites adhered for more than 4 hours to macrophages. Although they displayed high motility, ability to penetrate the cell membrane of the potential host cell was reduced. qPCR results confirmed that significantly less *T. gondii* entered *E. tenella*-activated MMs at 2 hpi, and a reduced proportion of intracellular *T. gondii* survived and replicated in these cells at 24 hpi.

Conclusions: We conclude that *E. tenella* modulates host cell responses to another apicomplexan agent, *T. gondii*, reducing active invasion and multiplication in chicken primary macrophages.

Background

Apicomplexan protozoa are obligate intracellular parasites causing a variety of diseases in animals and humans. *Toxoplasma (T.) gondii* infects almost all euthermic animal species and may also infect humans. About one third of the global human population are supposed to be infected by this zoonotic pathogen [1]. Chickens are considered to be an important reservoir of *T. gondii*. Especially free-ranging and back-yard chickens have been reported to show high seroprevalence rates for *T. gondii* [2]. On the other hand, coccidiosis caused by *Eimeria* species is a widely distributed major parasitic disease of poultry, one of the most pathogenic species in chickens being *E. tenella*.

Upon adherence to the host cell membrane apicomplexan parasites start to penetrate into the host cell by forming a so-called moving junction initiated by organelles of the apical complex at the anterior end of the parasites [3]. This moving junction supports the establishment of the parasitophorous vacuole (PV) separating the parasite from the host cell cytoplasm. The adhesive proteins produced by micronemes located in the apical region of the invasive parasite stages allow gliding motility which is essential for the active invasion by *T. gondii* [4]. In chickens, macrophages play a crucial role in identification and phagocytosis of pathogens including protozoan thus serving as a first line of innate immune defence [5]. However, *T. gondii* tachyzoites are capable to actively invade macrophages which occurs even faster than

phagocytosis displayed by a macrophage [6]. *T. gondii* tachyzoites are able to replicate in chicken blood monocyte-derived macrophages [7]. In experimental infection of *E. tenella* in naïve chicken, sporozoites were situated mainly within or next to the lamina propria that was infiltrated with macrophages in response to the infection [8]. Sporozoite-bearing macrophages are able to transport *Eimeria* sporozoites to the proper site of the intestinal mucosa [9]. However, *E. tenella* survival and development is rather poor in cultured chicken macrophages [10].

Concomitant infections by protozoan parasites and other microorganisms attract increasing attention in animals and humans both under natural conditions and in experimental *in vivo* and *in vitro* studies. For instance, probiotic bacteria reduced oocyst excretion of *E. acervulina* in chicken [11] as well as *in vitro* invasion of *E. tenella* into Madin-Darby bovine kidney cells [12]. Host immune responses and consequently clinical signs of disease are modulated in co-infected animals. In general, the immune response is dominated by Th1 during protozoan infection whereas Th2 response is typical for helminth infection [13]. Regarding interaction during co-infection by protozoa, it was reported that no competitive effects exist in mixed *Eimeria* species infection [14]. However, *T. gondii* replication was increased during co-infection with *Trypanosoma* in rats [15]. On the other hand, *T. gondii* supports *Plasmodium* replication in a rat model [16]. Natural co-occurrence of *T. gondii* and *Eimeria* in the same host has been reported. For example, both parasites were found concurrently in blood and organs of a heavily diseased sparrow [17]. Furthermore, wild rabbits tested seropositive for both *T. gondii* and *Eimeria stiedae* infection in Scotland [18].

In spite of high seroprevalence rates reported for both *T. gondii* and *E. tenella* in chickens, little is known to date about the mutual interplay between these two parasites, particularly during host cell invasion. Experimental *in vivo* and *ex vivo* co-infection models in chicken macrophages were established recently and thus suitable tools are now available for further co-infection studies [19, 20]. Results demonstrated that the mutual interaction during co-infection modulated both parasite replication as well as the host immune response. In our recent study, macrophage phagocytosis was distinctly altered during *in vitro* co-infection by *T. gondii* and *E. tenella* (unpublished data).

In the current study, we used live-cell imaging of *T. gondii* and/or *E. tenella* infected cells to monitor parasite invasion and survival at the single cell-parasite level in both mono-infected and co-infected cultures. The study focuses on the period of early parasite invasion in monocyte-derived chicken macrophages. A previous study showed that asexual stages of *T. gondii* and *Eimeria* displayed ultrastructural similarities [21]. Moreover, host cell invasion mechanisms, micronemal adhesins, gliding motility of both *T. gondii* tachyzoites and *Eimeria* sporozoites were identified to be affected *in vitro* by the same inhibitor of protozoan cGMP-dependent protein kinase [22]. Thus, we are particularly interested in whether the considerable capacity of *T. gondii* to invade and survive in macrophages is affected by host-specific *E. tenella* infection. Parasite tracking was performed to determine the motility of *T. gondii* during the invasion phase following co-infection by *E. tenella*. The life span of individual *T. gondii* was also monitored by live cell imaging.

Methods

Parasites and host cells

Tachyzoites of *T. gondii* RH - green fluorescent protein (GFP) strain (kindly provided by Professor Dominique Soldati-Favre, University of Geneva Medical School, Switzerland) were maintained at 37 °C with 5% CO₂ in human foreskin fibroblast (HFF) cells. Free tachyzoites were collected from the culture medium for infection. The sporozoites of *E. tenella* Houghton - yellow fluorescent protein (YFP) strain (kindly provided by Prof. Dr. Suo, China Agricultural University, China) were obtained from oocysts by excystation following an established protocol [23].

The animal experiments performed to collect chicken blood samples were approved by the responsible authorities (Landesdirektion Sachsen, Germany, trial registration number V13/10). Chicken peripheral blood mononuclear cells (PBMCs) were isolated from heparinized whole blood of adult chickens according to the established protocols with slight modifications kindly provided by Dr. Braukmann, Friedrich-Loeffler-Institute Jena, Germany. Briefly, PBMCs were separated from 2 ml blood by centrifugation (250 x *g*, 45 min) with Biocoll (density 1.077 g/ml; Biochrom AG, Berlin, Germany). Isolated PBMCs were resuspended and washed in 5 mL PBS (centrifugation 350 x *g*, 30 min) following pre-warmed 5 mL RPMI-1640 medium (Sigma, Taufkirchen, Germany) (centrifugation 350 x *g*, 20 min). Afterwards, 5 × 10⁶ PBMCs/well were resuspended in 24-well-plates in RPMI-1640 with 5% chicken serum and 5% fetal bovine serum, penicillin (100 U/mL, PAA), streptomycin (0.1 mg/mL, PAA), and amphotericin B (0.0025 mg/mL, PAA). After 72 h incubation (41 °C, 5% CO₂) PBMCs were trypsinized by Biotase® (Biochrom, Berlin, Germany) at 37 °C for 30 min. Detached monocyte derived macrophages (MMs) were counted under the microscope. 10³ MMs were seeded in the microscope imaging chamber (micro-insert 4 well, Ibidi, Martinsried, Germany) for 24 h (41 °C, 5% CO₂) for live cell imaging. For parasite quantification by qPCR, approximately 10⁵ MMs were seeded to 24-well-plates for 24 h (41 °C, 5% CO₂). The MMs were purified by rinsing off non-adherent cells once at 24 h and twice before infection.

Infection

Two experiments were conducted in this study (Fig. 1): Experiment 1 was designed to visualize parasite invasion; Experiment 2 was performed with increased MMs population at the same infection ratio to quantify parasite stages by qPCR for the different infection groups.

Study design for experiment 1 (Fig. 1A):

For imaging, cell cultures were assigned to four groups (n = 2 per group). In co-infected group CI, MMs were exposed to 2 × 10³ *E. tenella* sporozoites 2 h before *T. gondii* infection (-2 hpi). Group LPS cultures served as positive controls and were stimulated at the same time with 1 µg/mL lipopolysaccharide (LPS). Cultures of group Tg (monoinfection with *T. gondii*) and group NC (negative control) were not exposed to

LPS stimulation. At 0 hpi cultures of groups CI, LPS, and Tg were infected with 2×10^3 *T. gondii* tachyzoites per well. Group NC remained uninfected. All cultures were observed until 20 hpi. The whole experiment was repeated once biologically.

Study design for experiment 2 (Fig. 1B):

For quantification by qPCR, cell cultures kept in 24-well plates were assigned to five groups (n = 5 cultures/group). At -2 hpi, MMs of group CI (co-infection) and group Et (single infection with *E. tenella*) were exposed to 2×10^5 *E. tenella* sporozoites per culture. Group LPS cultures were pretreated with 1 µg/ml LPS at the same time. Group Tg was infected with 2×10^5 *T. gondii* tachyzoites per well at 0 hpi while group NC served as untreated, uninfected negative control. The cultures were maintained until 24 hpi.

Live-cell imaging of *T. gondii* in MMs

DRAQ7 dye was used to assess the viability of parasites and macrophages in each group. Prior to imaging, 3 µL DRAQ7 dye (Biostatus, Leicestershire, UK), a nuclear stain selective for dead cells, was added to all cell cultures immediately after exposure to *E. tenella* or LPS treatment (-2 hpi). The viability of parasites and cells were controlled by fluorescent microscopy prior to *T. gondii* infection at 0 hpi. Cells were viewed for fluorescence by confocal laser scanning microscope (CLSM) (TCS-SP8, Leica, Bensheim, Germany) using 4 channels at 488 nm, 514 nm, 633 nm laser line and wide field. Basic imaging parameters were 40 x objective, 10 x ocular (NA 0.90), 1024 × 1024 dpi, 6 Z-stacks (4 µm). To avoid crosstalk between channels, images were collected in line sequential mode.

Incubation conditions (41 °C, 5% CO₂ and 99% humidity) were controlled using an incubation chamber (Tokai-Hit, Shizuoka, Japan) over the whole observation period. The timeframe of infection and imaging is shown in Fig. 1A. Briefly, after adding DRAQ7, approximately 20 random fields of cells were selected and captured according to groups CI, LPS, and Tg from 2 individual wells per group. Image collection for time-lapse imaging of tachyzoite motility was performed at an interval of 10 min per frame until 2 hpi. Image acquisition was interrupted from 2 hpi to 3 hpi to add 20 additional fields per group which contained intracellular *T. gondii* in group CI and LPS. All fields were observed for further 4 h until 7 hpi at an interval of 30 min per frame. Subsequently, at least 100 cells with live intracellular *T. gondii* (1–2 tachyzoites / cell) were collected and captured at 7 hpi and 20 hpi by CLSM for groups CI and Tg. For group LPS, only 71 MMs with live intracellular *T. gondii* were selected due to a generally low number of *T. gondii*-positive cells observed in this group. Six fields were analysed randomly for group NC in parallel.

Images of stacks were obtained using LAS X software (Leica, Bensheim, Germany). Stacks were analysed with Imaris® software version 9.3 (Bitplane, Abingdon, UK) using functions of spot detection and tracking parasite motility and viability.

Parasite Quantification By Quantitative Real-time Pcr (qpcr)

For all infection groups (Fig. 1B), complete cell populations from a subset of cell culture wells were collected at 2, 6, 12, and 24 hpi and additionally at 0 hpi in group Et. DNA was extracted using the QIAamp DNA Mini Kit® (Qiagen, Hilden, Germany) following the manufacturer's instructions. qPCR was performed using the CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, USA). Data represent the mean of three replicates with an acceptable standard deviation of less than 0.5 for Ct values.

T. gondii multiplication was analyzed by a probe-based qPCR detecting the 529-bp repeat element [24]. Standard curve was developed by data obtained for gradient 10-fold dilutions of initially 10^7 tachyzoites. qPCR was conducted in a total volume of 25 μ L: 5 μ L of sample DNA, 12.5 μ L of Master Mix, 3.2 μ L of DNase/RNase free water (Gibco™, Life Technologies, USA), 2.5 μ L of 2 μ M TaqMan probe and 0.9 μ L of each 25 μ M forward and reverse primer (5'-CACAGAAGGGACAGAAGT and 5'-TCGCCTTCATCTACAGTC-3'). The cycling program consisted of 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 15 s.

ITS1 fragment quantification was used to assess the replication of *E. tenella* by a SYBR Green-based PCR [25]. The relative copy number of *E. tenella* DNA was implemented by measurement of pSCA-17 plasmid standard dilution as described before [26]. qPCR was conducted in a total volume of 20 μ L: 2 μ L of sample DNA eluate, 10 μ L of SYBR Green master mix (Thermo Fisher Scientific, Darmstadt, Germany), 7.2 μ L of water, 0.9 μ L of 25 μ M forward and reverse primer (5'-aacctgactgtgcaagcatc-3' and 5'-atcatagacagccgtgcccag-3'). The cycling program consisted of heating to 95 °C for 5 min, followed by 40 cycles at 95 °C for 30 s, 55 °C for 20 s, and 72 °C for 20 s. A subsequent melting curve analysis (95 °C for 1 min, 55 °C for 30 s, 0.5 °C/s) was performed to create the dissociation curve.

Statistical analysis

Statistical analysis was performed by using Imaris® and GraphPad Prism® (version 8, San Diego, CA, USA) software. Kolmogorov-Smirnov test was performed to test for normal distribution. Statistical significance was assessed by two-way ANOVA for data with normal distribution and Tukey's multiple comparisons test for values that did not follow normal distribution.

Results

Live cell imaging of *T. gondii* in MMs

In general, penetration of *T. gondii* tachyzoites into the MMs started within 2 hpi after tachyzoites were seeded into cultures. CLSM analysis (Fig. 2A) of group CI cultures showed that *T. gondii* tachyzoites remained loosely adherent to MMs for more than 4 hours in most cases before they started to actively invade the host cell. During this phase, distinct helical gliding motility of tachyzoites was seen. This

untypical behavior of tachyzoites in group CI was observed regardless whether intracellular *E. tenella* sporozoites were still alive or dead. An additional movie file shows this in more detail (see Additional file 1). In contrast, tachyzoites, which were actively or passively incorporated into MMs after attachment, were seen within minutes in the mono-infected group Tg and the LPS-treated group.

Tachyzoites showed lowest motility in group LPS at most of the captured time points (Fig. 2B). In group CI, significantly higher motility of tachyzoites were observed than in group LPS over the period from 10 min to 120 min post infection ($p < 0.05$); with the exception of 70 to 110 min. Comparing between groups CI and Tg, tachyzoites of group CI showed significantly higher motility ($p < 0.05$) during the first 20 min. Average motility values in group CI tended to remain higher than in both groups Tg and LPS with a significant difference ($p < 0.05$) at 80, 90 and 110 min compared to group Tg.

Parasite Quantification

In experiment 1, the relative intensity (RI) of green fluorescence of 27 to 30 individual intracellular *T. gondii* tachyzoites which were captured at 3 to 7 hpi by CLSM were analysed in all infected groups. RI revealed no significant difference between groups Tg and CI until 4 hpi. Thereafter, RI values remained on a consistently higher level in group CI than in the monoinfected group Tg and in group LPS (Fig. 3A). The RI measured in group CI displayed only slight variation from 3 to 7 hpi, whereas values started to decrease in group LPS and Tg following 4.5 hpi or 5 hpi with a statistically significant difference between groups CI and LPS ($p < 0.05$) at 4.5 hpi.

In general, less than 45% of traced intracellular tachyzoites of *T. gondii* were still alive or replicating in MMs at 20 hpi (Fig. 3B). At 20 hpi, the proportion of *T. gondii*-positive MMs was lower in group CI (27%) than in group Tg (45%) and only slightly higher than in group LPS (21%).

By qPCR, significantly lower DNA copy numbers ($p < 0.05$) were determined at 2 hpi for *T. gondii* in group CI and group LPS compared to group Tg (Fig. 3C, left). The number of DNA copies remained on a low level in the two former groups until 12 hpi, followed by a steep increase until 24 hpi in group CI while values remained significantly lower in group LPS ($p < 0.05$). In group Tg, DNA copy numbers were higher than in the two other groups at 2 hpi, decreased steadily until 12 hpi to a level similar to groups CI and LPS and increased even more distinct than those recorded for group CI thereafter. No statistical difference was observed between group CI and Tg at this time point ($p > 0.05$). Likewise, no significant difference in DNA copy numbers was detected for *E. tenella* by comparison of groups Et and CI over the entire observation period of 24 hpi (Fig. 3C, right).

Discussion

After two hours of exposure to *E. tenella* sporozoites or LPS MMs generally maintained their viability which was demonstrated by negative staining for DRAQ7 (data not shown). A recent study by [20], confirmed earlier findings by [27] that *E. tenella* sporozoites were mostly located within chicken

macrophages at 2 hpi. In the current study, most intracellular *E. tenella* sporozoites showed red fluorescence after DRAQ7 staining instead of the expected yellow (YFP) fluorescence within 4 hpi to 6 hpi (data not shown), indicating their death. This CLSM finding corroborates the qPCR results demonstrate that the number of *E. tenella* declined to lowest numbers at 6 hpi (Fig. 3A, right). Co-infection with *T. gondii* did not exhibit a significant influence on *E. tenella* replication when compared to single *E. tenella* infection. Unfortunately, we could not quantify the *E. tenella* sporozoite numbers via YFP through time lapsing imaging because of a non-ideal YFP expression (about 80%) in the available parasite strain.

Host cell invasion by *T. gondii* tachyzoites usually takes only 15 to 20 s [6], 1995). It is assumed that *T. gondii* tachyzoites and *Eimeria* sporozoites may invade and traverse several host cells by disrupting the host cell membrane [28]. In our time lapsing study, *T. gondii* tachyzoites were either floating free in the culture medium (unattached to macrophages) for hours or displayed a rapid entry into the macrophages, in both group LPS and group Tg. Interestingly, adherence of vital tachyzoites was prolonged for more than 4 hours in the co-infection cultures. It was previously demonstrated that most *T. gondii* tachyzoites remained adherent to murine macrophages that were treated with a phagocytosis inhibitor, Cytochalasin D [29]. Microneme exocytosis is necessary for host-cell entry of both *T. gondii* [30] and *E. tenella* [31]. The micronemes appear to be both structurally and functionally conserved between different members of the Apicomplexa [4]. We assumed that *E. tenella* infection may potentially hamper the entry of *T. gondii* into host cells by altering recognition of signal receptors or inhibiting phagocytosis.

Rapid invasion and egress are crucial to *T. gondii* survival and successful replication, thereby minimizing the exposure to destructive reaction by innate protection in a generally hostile extracellular environment [32]. Once inside the host cell, the parasite no longer moves [33]. The intracellular survival of *T. gondii* depends on the route by which the parasite enters the host cell. Although *T. gondii* tachyzoites are phagocytized and internalized through Fc receptor mediation [34], intracellular *T. gondii* can survive and replicate within their PV by blocking the host macrophage's pathways intended to initiate vacuolar acidification and parasite inactivation [35]. To quantify viable intracellular *T. gondii*, the intensity of GFP signal expression by the parasite was monitored in this study. It appeared from our observations according to RI values that *T. gondii* showed better tolerance and survival at the early stage of infection (until 7 hpi) if MMs were previously exposed to *E. tenella* (Fig. 3A).

Under the conditions of our experimental design (two *T. gondii* tachyzoites per cell), most infected host cells contained only one parasite at 7 hpi in all infected groups. Compared to group LPS, By CLSM, only slightly high *T. gondii*-positive cells were monitored compared to group LPS. However, significant high number of total DNA copies of *T. gondii* was detected in group CI compared to group LPS. Therefore, we conclude that the single cell observation by CLSM may not be completely comparable with quantification of DNA copies in cell cultures due to the different amount of cells considered. Nonetheless, results obtained with both methods indicated a reduced growth of *T. gondii* in chicken macrophages in a co-infection setting with *E. tenella* compared to mono-infection.

Macrophages are not only professional phagocytes but also secrete cytokines in response to parasite infection. This innate immune response can be triggered in chicken macrophages by exposure to LPS [36]. A previous study showed that *T. gondii* blocked LPS-induced production of IL-12 and TNF-alpha in murine bone marrow derived macrophages [37]. For *E. tenella*, it is known that IL-1 β and iNOS expression are significantly enhanced in chicken HTC macrophages by merozoites at 2 hpi [38]. A recent *in vitro* study demonstrated down-regulation of IL-12 and iNOS in chicken macrophages during simultaneous co-infection by *T. gondii* and *E. tenella* [20]. From previous as well as the current data it appears that modulation of innate immunity in chicken differs during mono- and co-infection, which includes cytokine production and macrophage phagocytosis. However, data on concurrent infections with *E. tenella* and *T. gondii* are still scarce although both are considered to be common pathogens in poultry and thus deserve more attention.

Conclusions

In summary, live cell imaging by CLSM proved to be a useful tool to evaluate chicken macrophage invasion and / or phagocytosis during mono- and co-infection with two different apicomplexan parasites. It was demonstrated that the mechanisms of *T. gondii* invasion and survival appear to be altered in *E. tenella*-exposed macrophages. Further studies into macrophage signaling pathways, particularly modulation of the cytokine response, combined with image analysis and live cell imaging, will help to better understand the function and modulation of the innate immune response during apicomplexan invasion.

Abbreviations

PV: parasitophorous vacuole; GFP: green fluorescent protein; YFP: yellow fluorescent protein; PBMCs: peripheral blood mononuclear cells; LPS: lipopolysaccharide; CLSM: confocal laser scanning microscope;

Declarations

Ethics approval and consent to participate

The animal experiments performed to collect chicken blood samples were approved by the responsible authorities (Landesdirektion Sachsen, Germany, trial registration number V13/10)

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' Contributions

RZ designed the study, wrote the manuscript, and performed the experiments and data analysis. WZ helped to perform cell imaging and manuscript drafting. AD and BB critically revised the study design, data interpretation and manuscript. All authors read and approved the final manuscript.

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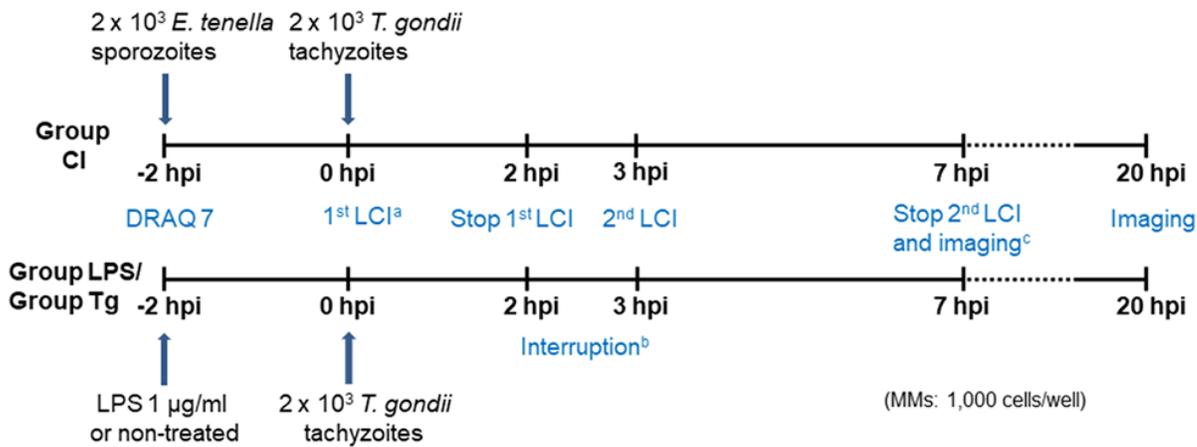
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Additional File 1

Video microscopy of *T. gondii* invasion in a co-infected cell with an *E. tenella* sporozoite at 0-4.5 hpi. *T. gondii* is adherent on the cell which contains a vital *E. tenella* sporozoite. Yellow fluorescence: the nucleus of *E. tenella* sporozoites; green fluorescence: *T. gondii* tachyzoites; wide field: single macrophage.

Figures

(A) Experiment 1



- ^a Live cell imaging (LCI)
- ^b 1 hour Interruption to include new imaging fields of intracellular *T. gondii*
- ^c single cells with intracellular *T. gondii* were selected and captured by CLSM

(B) Experiment 2

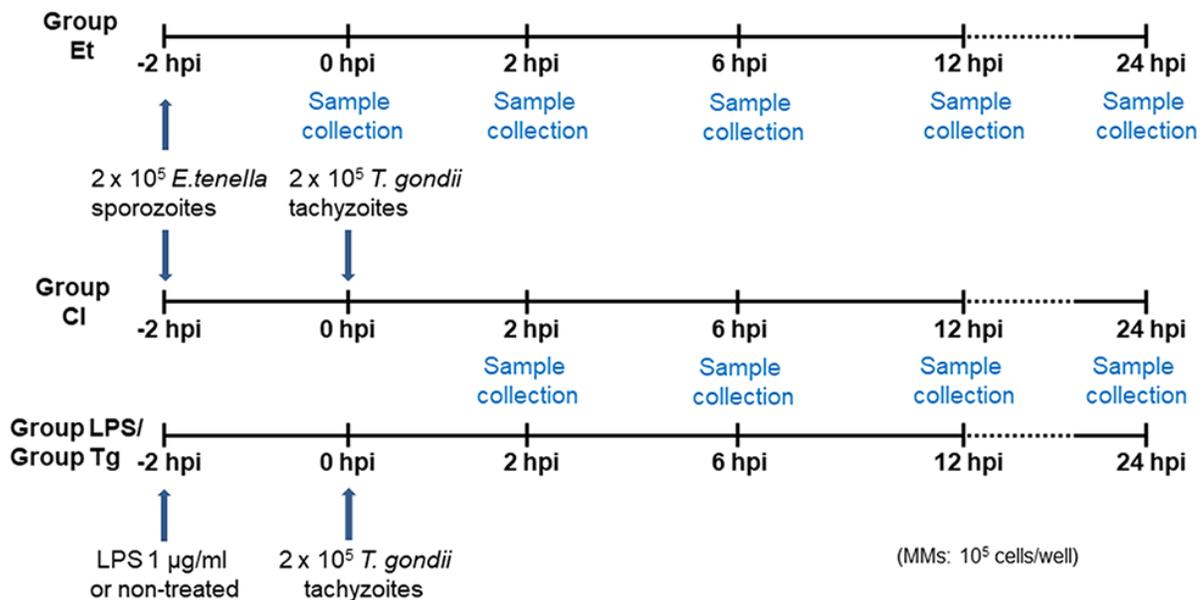


Figure 1

Schematic representation of infection and imaging. (A) Experiment 1 (live cell imaging). Images of group NC were collected at the same time points as in groups CI, LPS and Tg. (B) Experiment 2 (parasite

quantification by qPCR). Samples of group NC were collected at the same time point as in groups CI, LPS and Tg.

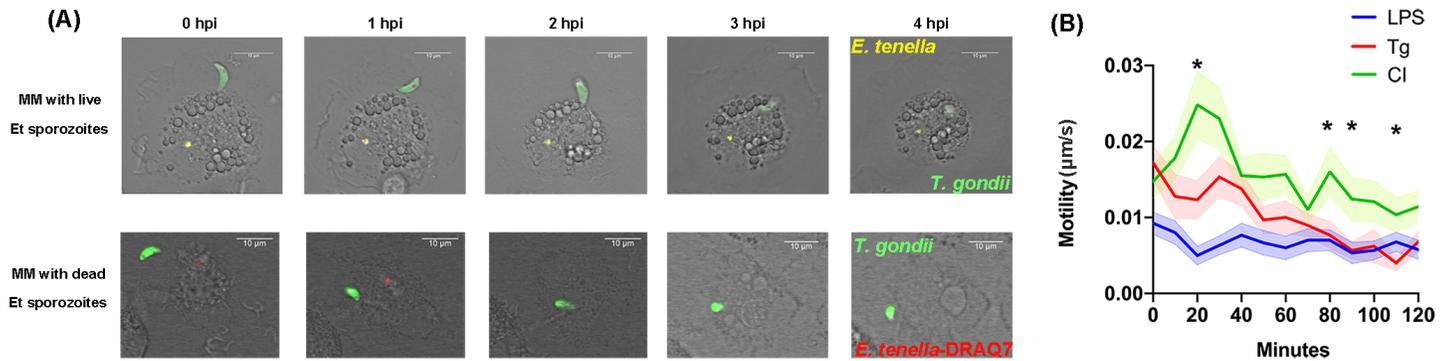


Figure 2

Invasion of *T. gondii* within 4 hpi. (A) Video microscopy of *T. gondii* invasion at 0-4 hpi in a cell previously exposed to *E. tenella*. (a) *T. gondii* is adherent on the cell which contains a vital *E. tenella* sporozoite. (b) *T. gondii* is adherent on the cell which contains a dead *E. tenella* sporozoite that was phagocytized and fused by macrophages at 2 hpi. Yellow fluorescence: the nucleus of *E. tenella* sporozoites; green fluorescence: *T. gondii* tachyzoites; wide field: single macrophage. (B) Motility (speed) of live *T. gondii* tachyzoites (0-120 minutes). The motility of *T. gondii* tachyzoites (n=30) was assessed by imaging every 10 minutes over 120 minutes. Each parasite was tracked over 70-100% of all time points excluding the occasional out-of-range movement or parasite death. Mean values per time point were calculated. LPS: LPS-treated, *T. gondii* mono-infection; Tg: *T. gondii* mono-infection; CI: co-infection. *p < 0.05, CI compared to Tg; Error bar: standard error of the mean (SEM)

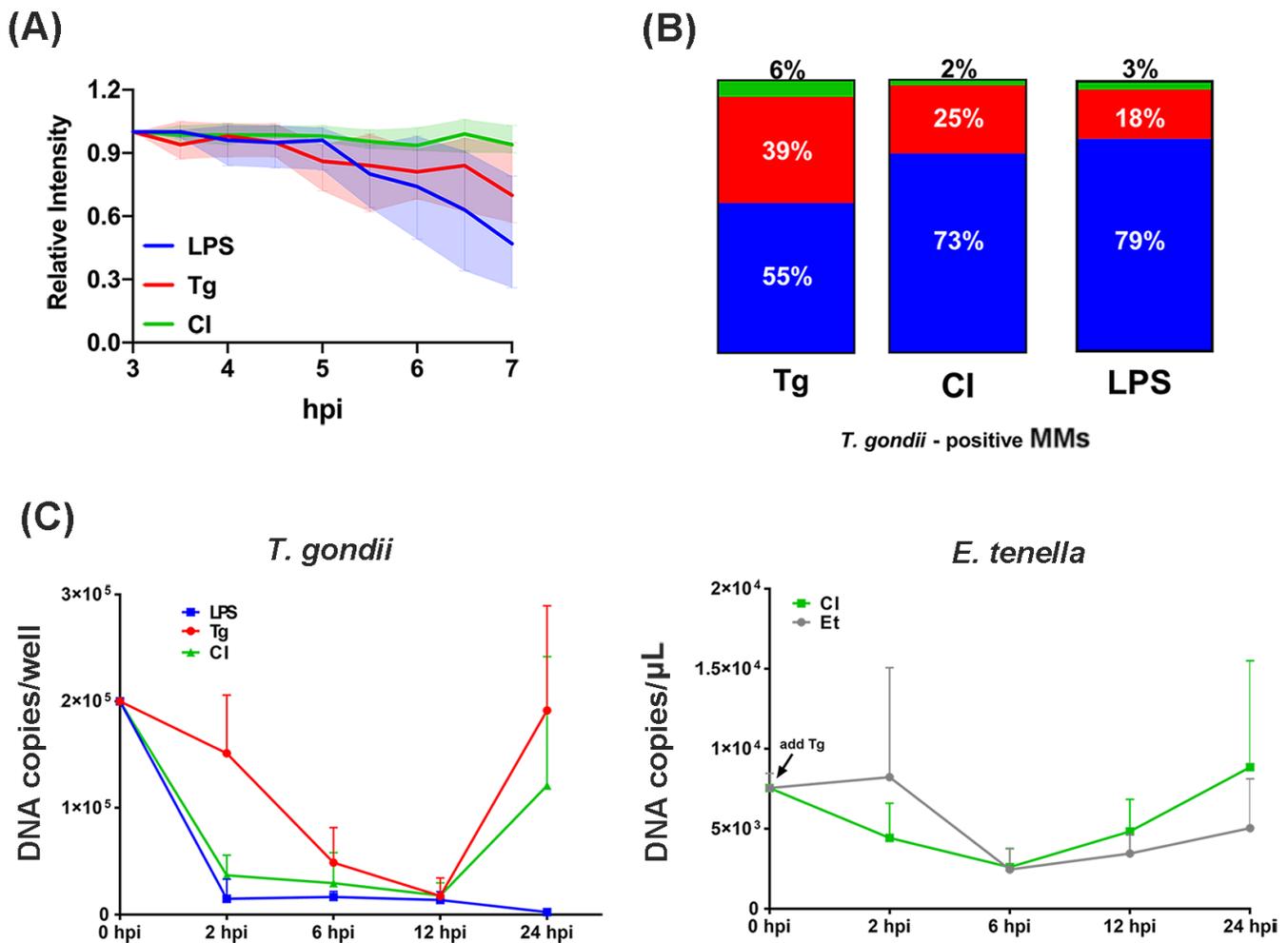


Figure 3

Quantitative analysis of *T. gondii* (A) Assessment of intracellular survival of *T. gondii* (n=27-30) by measurement of Relative Intensity (RI) at 3-7 hpi. RI = initial mean intensity of fluorescence for each parasite / mean intensity of fluorescence for each parasite per time point. RI = 0 means no signal of fluorescence is detected. Error bar: standard error of the mean. (B) *T. gondii* negative/positive MMs. 100 vital *T. gondii*-positive MMs in group CI and Tg and 71 MMs in group LPS (due to low numbers of surviving parasites) were selected and marked randomly, i.e. 1-2 vital intracellular tachyzoites / cell were captured at 7 hpi and again at 20 hpi. The rate of *T. gondii*-negative/positive cells was calculated. Blue column, MMs with reduced numbers of or non-vital *T. gondii*; Red column, MMs with constant numbers of vital *T. gondii*; Green column, MMs with replicating *T. gondii*. (C) Parasite quantities (DNA copies) during sequential co-infection. Parasite replication is represented as mean value with standard deviation (n=3-5 per time point). LPS: LPS-treatment, *T. gondii* mono-infection; Tg: *T. gondii* mono-infection; Et: *E. tenella* mono-infection; CI: co-infection.

Supplementary Files

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